Vacuum freeze-drying effect on bioactive compounds of eggplant (Solanum Melongena L.).

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# VACUUM FREEZE DRYING EFFECT ON BIOACTIVE COMPOUNDS OF EGGPLANT (*SOLANUM MELONGENA L.*)

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***Abstract***
*Vacuum Freeze Drying (VFD) is a low temperature drying technique that may be used for food preservation. The aim of this work is to evaluate how VFD operating conditions affect eggplants bioactive compounds loss after drying. Samples were freeze-dried under different pressure and temperature conditions, and had their ascorbic acid, total polyphenol and antioxidant capacity percent loss measured after processing. Under the tested conditions, lower temperatures resulted in lower antioxidant capacity in the final product, while lower chamber pressures resulted in lower total polyphenol content.*

***Keywords**: vacuum freeze-drying, eggplant, ascorbic acid, antioxidant capacity, total polyphenol content.*

## 1. Introduction

Fruits and vegetables usually have a high-water content, favoring microbial growth and several biochemical reactions which may lead to undesirable changes in the product. For this reason, dehydration is an important food preservation method (Fissore and Velardi 2012, Orphanides *et al.* 2016). However, drying may partially or severely affect the properties of the product, especially when high temperatures are used. Freeze-drying is an advanced low temperature dehydration process based on the sublimation of ice as the water removal mechanism. This sublimation may happen under vacuum (VFD) or atmospheric pressure (AFD) since the driving force is the vapor pressure gradient between the food product and the chamber (Berk 2013, Meryman 1959, Claussen *et al.* 2007).

Freeze-drying typically has a lower impact on the nutritional and physical properties of food products compared to other drying methods (Fissore and Velardi 2012, Berk 2013). Seasonal, perishable and highly nutritious food products are good candidates for preservation through dehydration methods and more advanced processing methods, such as VFD, may be worth to be applied to preserve their properties (Ratti 2001).

Drying time is an important issue in freeze-drying processes since it is usually longer compared to other drying methods. In VFD, the thermal exchange between the equipment and the product may be affected by the low pressure, in particular the conduction between the heating shelf of the freeze-dryer and the product lying upon it. In AFD, the diffusion of water vapor in the solid matrix is usually the rate controlling process. Accordingly, it is critical to understand how the operating parameters affect the process rate.

Additionally, product temperature, chamber pressure and processing times are variables that may affect the nutritional compound loss of foods during a freeze-drying process and thus, this should be further investigated. Eggplant (*Solanum melongena L.*) is a very interesting case study since it exhibits

a high antioxidant capacity. In addition, it has a soft porous structure which requires shorter freeze-drying times and, thus, it is a good candidate for obtaining commercial freeze-dried products requiring short drying times (García-Salas *et al.* 2014).

The objective of this study is to evaluate the effect of the operating conditions (shelf temperature and chamber pressure) of the drying step on targeted nutritional properties (vitamin C, total phenolic content and antioxidant capacity) of eggplants (*Solanum melongena L.*) using VFD. Colucci *et al*. (2018) studied the effects of AFD on the same bioactive compounds on black beauty eggplants using similar materials and methods. As a representative evaluation of the effects of both processes, the results of this present study will be briefly compared to those obtained by Colucci *et al*. (2018).

## 2. Material and method

The freeze-drying experiments were carried out in a LyoBeta 25 by Telstar (Terrassa, Spain), which is a pilot-scale equipment with a 0.2 $m^3$ chamber. It has an external condenser which operates at approximately -80°C (having a maximum ice capacity of 40 kg). The ratio between the capacitance gauge (Baratron type 626A, MKS Instruments, Andover, MA, USA) and a thermal conductivity gauge (Pirani type PSG- 101-S, Inficon, Bad Ragaz, Switzerland) measurements was used to outline primary drying duration. In fact, the ratio between the Pirani and the capacitive pressure measurements present a sharp decreasing trend as the drying process is close to be completed (Patel *et al.* 2010). Accordingly, to define a representative time for the end of primary drying, the midpoint of the slope was regarded as the average primary drying duration. In addition, to consider process variability, the point immediately prior to the sharp pressure-ratio slope was set as the onset time and the point immediately after, the offset time. To monitor the samples surface temperature throughout the process, the infrared thermography-based sensor (FILR A35) presented by Lietta *et al.* (2018) was used.

The effect of the two factors, temperature and pressure, was evaluated in a $2^2$-factorial design with a central point. Every factor was tested at two levels, high and low: temperature at −30°C and 0°C and pressure at 10 and 30 Pa; the central point was -15°C and 20 Pa. Each drying condition was tested one time, with an additional repetition for the central point to ensure statistical significance of the results (Montgomery C. 2001). To evaluate if there was any significant difference between the average drop in the concentration of ascorbic acid, total phenolics content and antioxidant capacity measured after each of the tested processing conditions, an analysis of variance (ANOVA) was done using MiniTab® 17. Factors were considered significant when the p-value was lower than 0.05.

Eggplants (*Solanum Melongena L.*) of the black beauty variety were purchased every morning of experiments from the same local vendor and were used for the tests. All samples tested were cut in 9 mm side cubes, pre-treated with sodium metabisulfite solution at 2% w/w (Honeywell Fluka™, ≥ 98%). Since samples were pretreated with sodium metabisulfite, the concentration reduction of the targeted compounds after drying was estimated by comparing the initial concentration ($c_0$) of the components in the pre-treated fresh samples to that after freeze-drying ($c_d$). The percentage component loss after drying was calculated as described in Equation 1.

$$\%\ reduction = \frac{c_0 - c_d}{c_0} \times 100\% \qquad (1)$$

The extraction of the bioactive compounds from the eggplants was done with ethanol (Honeywell Fluka™, 96% v/v) at room temperature (20°C). Each extraction was performed by following the same procedure, using 1 ± 0.05 g of dried product for freeze-dried samples, or 16 ± 0.15 g of fresh eggplant for fresh pretreated samples, which represented approximately 28 cubes for each extraction. These sample cubes and 30 mL of ethanol were smashed with a mortar and pistil, then homogenized with an Ultra-Turrax® (IKA T-25) for 3 minutes at 9500 rpm, and, finally, put into a magnetic stirrer for 20 minutes. The final extract was obtained by light vacuum filtration, performed with a vacuum pump (BUCHI V-700) and glass microfiber filter (GFFC grade, 1.2 µm). The final volume of the extract was adjusted in a 50 mL volumetric flask. During the extraction the product was protected from light

degradation by covering the container with an aluminum foil, and the analytical assays to evaluate the final product nutritional properties were carried right away after extraction. For each tested condition, two extract replications were done. For each extract, all spectrophotometric measurements (JENWAY, 6850 UV/Vis) were done also in triplicate, and the average results found for each sample were used for the statistical analysis.

*Ascorbic acid content (AA)*

The method used to measure the ascorbic acid content was a colorimetric test, based on (Jagota and Dani 1982), using the following reactants:

- Folin-Ciocalteu (Sigma-Aldrich, 2 M) reagent, diluted in distilled water (1:10 v/v);
- Trichloroacetic acid (Sigma-Aldrich, 6.1 N), at 7.5% by volume.

At first, 1 mL of extract and 1 mL of trichloroacetic acid were vigorously mixed in a cylinder and then left to rest for 5 minutes in a fridge at 4±1°C. Then, the solution was filtered with a 0.45 µL nylon syringe filter (SFNY, 0.45 µm). Both for the measures on the fresh and on the dried product it was not necessary diluting the extract. For the colorimetric reading, 0.2 mL of this filtered extract were placed in a 4.5 mL spectrophotometer cuvette with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of water. After 10 minutes resting in the dark, the absorbance was read at 720 nm, at its peak absorbance.

The amount of vitamin C was quantified through a calibration curve previously obtained with known solutions of ascorbic acid and water, in the range of 25 – 600 $mg_{AA}.L^{-1}$. The results were reported as equivalent milligrams of ascorbic acid ($mg_{AA}$) per gram of sample on wet basis ($g_w$).

*Total phenolic content (TPC)*

The method used was based on (Gao *et al.* 2000), with some modifications as used by Colucci *et al*. (2018). The reactants used in this case were the following:

- Folin-Ciocalteu (Sigma-Aldrich, 2 M) reagent, diluted in distilled water (1:10 v/v);
- Sodium carbonate (Chem Lab NV, 99.8%), at 20% w/v.

In order to be in the adequate range for measurement, the extract was firstly diluted with ethanol (Honeywell Fluka™, 96% v/v). The extracts made from the fresh eggplant were diluted 1:9 v/v, while those obtained from the dried product 1:4 v/v. In a 4.5 mL spectrophotometer cuvette 0.1 mL of the diluted extract was placed, with 0.2 mL of Folin-Ciocalteu reagent and 2 mL of water. After three minutes in the dark, 1 mL of sodium carbonate was added to each cuvette. After 1 h of incubation in the dark, at room temperature, the absorbance was read at 765 nm.

The calibration curve to measure the content was obtained with gallic acid (Sigma-Aldrich, 98%) and ethanol (Honeywell Fluka™, 96% v/v) in the range of 20 – 120 $mg_{GA}.L^{-1}$. The results were reported as milligrams of gallic acid ($mg_{GA}$) per gram on wet basis.

*Antioxidant capacity (AC)*

To determine the antioxidant capacity of the extracts, the assay used was the Ferric Reducing Antioxidant Power (FRAP) as done by Colucci *et al*. (2018) and the reactants used were the following:

- Iron (III) chloride.6aq (LabChem NV, 99%);
- Glacial acetic acid (LabChem NV, 99-100%);
- Sodium Acetate (Honeywell Fluka™, ≥ 99%);
- 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, 99%);
- HCl 40 mM (Sigma-Aldrich, 37%).

The assay is based on a mixture of equal volumes (1:1:1) of the following three solutions: an acetate buffer 0.3 M, pH 3.6 (0.155 g of sodium acetate and 0.8 mL of acetic acid in distilled water), a

20 mmol L-1 ferric chloride solution (0.2717 g in 50 mL of water), and a TPTZ solution (0.064 g in 20 mL of HCl at 40 mM). These three reactants were prepared in the same day of the tests and stored at room temperature, protected from light. The extract from the fresh eggplant was diluted 1:19 v/v, and that from the dried product 1:7 v/v. 1.5 mL cuvettes for the spectrophotometric analysis were filled with 30 µL of diluted extract, 30 µL of water, and 900 µL of the FRAP mixture, then incubated in a 37°C thermal bath for 30 minutes before reading the absorbance at 595 nm.

The calibration curve to measure the antioxidant capacity was obtained with Trolox (Sigma-Aldrich, 97%) and ethanol (Honeywell Fluka™, 96% v/v) in the range of 60 – 200 $mg_T.L^{-1}$. The results were reported as milligrams of Trolox ($mg_T$) per gram on wet basis.

## 3. Results and discussion

*Influence of the operating parameters on drying time and product temperature*

The shelf heating temperature and chamber pressure influence drying times and product temperature, which in turn affect product quality. The time required to complete primary drying under different operating conditions, evaluated through the midpoint of the pressure ratio curve, is shown in Table 1. Chamber pressure seems to not expressively influence process duration, as the difference between the time required at 10 Pa and 30 Pa, both at high and low temperature, appears not meaningful. On the other hand, the effect of the temperature is much relevant, as the time required by the batches at 0°C is less than a half than that of the batches at -30°C. Indeed, the operational pressure under the tested conditions was found to not have a significant effect (p-value = 0.872) on drying time though a one-way ANOVA, while the temperature was significant (p-value = 0.002).

*Table 1. Representative primary drying average durations.*

| Operating conditions | | Primary drying |
|---|---|---|
| Shelf temperature, °C | Chamber pressure, Pa | time, h |
| -30 | 10 | 14.3 |
| - 30 | 30 | 13.6 |
| -15 | 20 | 7.2 |
| 0 | 10 | 5.6 |
| 0 | 30 | 4.3 |

For purposes of comparison, sample surface temperature profiles observed at different chamber pressures and shelf temperatures are reported in Figure 1. As for the drying time, pressure seems to have a small influence on the product temperature as shown by the trends at 0°C and different chamber pressures of 10 and 30 Pa. On the other hand, the surface temperature varied according to the shelf temperature, as expected. The thermal profile at -30°C and 30 Pa was very different from the one at the same pressure but at 0°C.

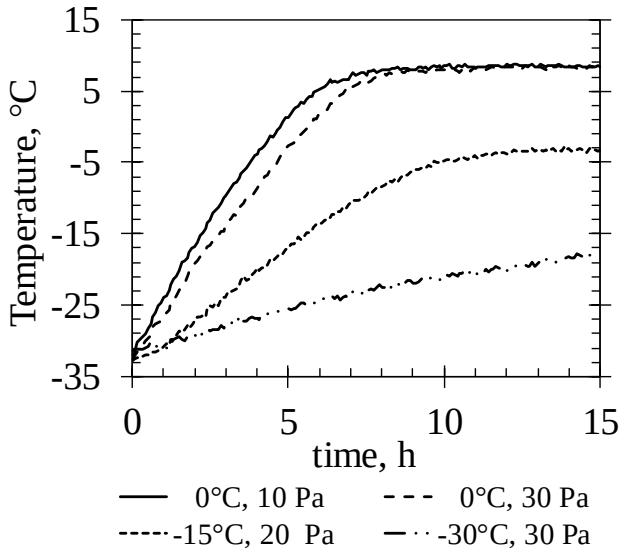


*Fig 1. Surface temperature profiles measured for different freeze-drying conditions*

*Influence of operating parameters on quality properties*

The operating parameters of the freeze-drying process, namely the temperature of the heating shelf and the pressure of the drying chamber, influence also the nutritional properties of the eggplants. Differences on the degradation of antioxidant compounds were found by (Herbig and Renard 2017) when operating under diverse temperature ranges, suggesting that different degradation mechanisms might be in place according to the operating conditions.

For the fresh eggplant, the average concentrations of the compounds of interest were: 0.07 ± 0.01 $mg_{AA}/g_w$ for ascorbic acid, 0.24 ± 0.06 $mg_{GA}/g_w$ for TPC and 0.63 ± 0.09 $mg_T/g_w$ for the antioxidant activity. Hanson *et al.* (2006) found ascorbic acid values ranging from 56 ± 14 $mg_{AA}$/100g to 129 ± 9 $mg_{AA}$/100g dry weight basis, which corresponds approximately to values from 0.03 $mg_{AA}/g_w$ to 0.08 $mg_{AA}/g$ wet weight basis (Hanson *et al.* 2006). Ninfali *et al.* (2005) reported TPC values expressed as 54.7 ± 6.0 mg of caffeic acid equivalents per 100 g in fresh weight basis for the black beauty variety (Ninfali *et al*. 2005). Finally, the antioxidant capacity found by Kaur et al. (2014) for eggplant variaties was from 0.72 ± 0.4 µmolTrolox/gw to 8.11 ± 1.2 µmolTrolox/$g_w$, which corresponds roughly to 0.2 mgT/$g_w$ to 2 mgT/gw (Kaur *et al.* 2014). It can thus be concluded that the concentration of AA, TPC and the AC of the fresh product considered in this study are in good agreement with the values reported in the literature (taking also into account normal product variability).

Regarding the quality properties of eggplants, under both chamber pressures, lower shelf temperatures seemed to promote an increase in compound loss as shown in Figure 2. This negative effect of lower temperature was found significant only for AC (p-value = 0.041). Higher AC loss at -30°C might be related to process duration, since samples processed under lower temperatures required longer drying-times which could favor compound loss, since it provides more time for degradation reactions to occur.

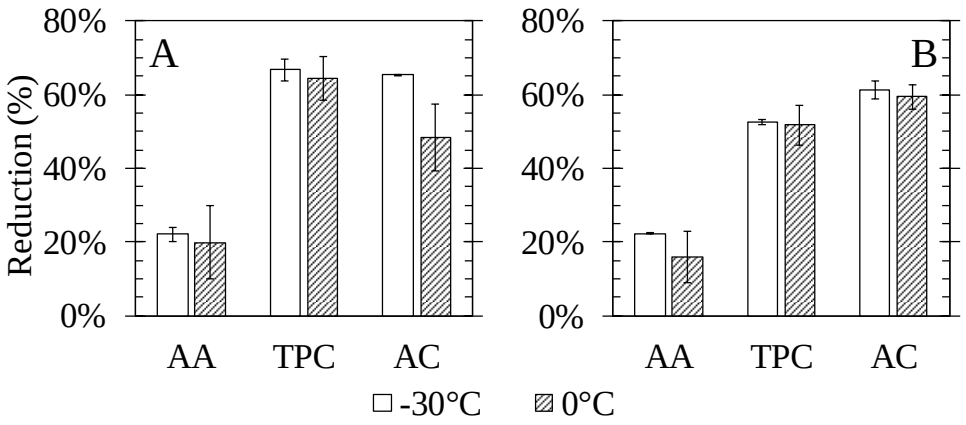


*Fig 2. Shelf temperature effect at 10 Pa (graph A) and at 30 Pa (graph B).*

Pressure had no significant effect neither for AA nor for AC percentage loss (p-values > 0.05). Still, pressure was found to have a significant effect on TPC loss (p-value = 0.006). From the data under the tested conditions, lower pressure resulted in higher TPC loss as presented in Figure 3. As previously stated, the operating pressure has a lower impact on the drying time. Thereby, the difference in TPC reduction found between batches operated at the same temperature, but different pressures, do not seem to be related to the process duration, but to the chamber pressure itself. This could be related to some effect on the volatization of measured compounds.

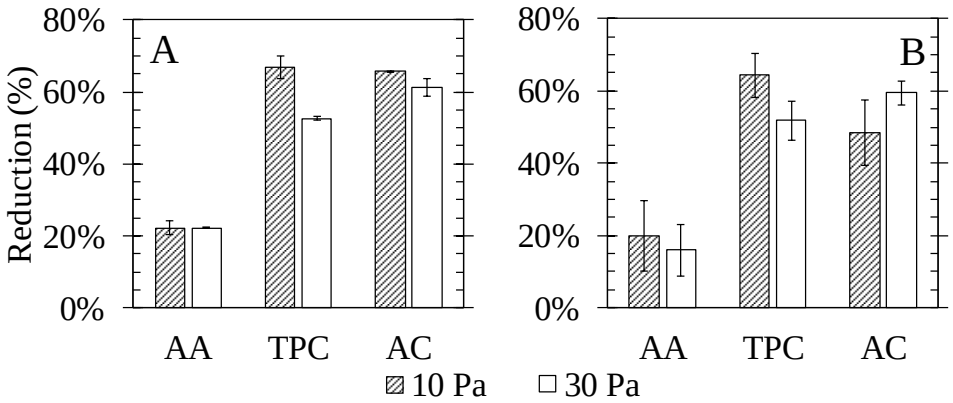


*Fig 3. Chamber pressure effect at -30°C (graph A) and at 0°C (graph B).*

*Comparison between AFD and VFD*

To compare the effects of AFD and VFD on the nutritional properties of eggplants, some results obtained by AFD, presented by Colucci *et al*. (2018), can be confronted to those obtained by VFD. Drying times for AFD varied from 11.0 ± 1.5 h for 7.5°C air temperature to 15.3 ± 0.9 h for -10°C. AFD ascorbic acid percentage reduction was 46±9% for -10°C air temperature, 63±10% for -7.5°C and 61±11% for -5°C. Total phenolic content reduction was 58±17%, 71±19% and 65±16% respectively for the same air temperatures. Finally, antioxidant capacity reduction, also by the FRAP assay, was 34±23%, 59±16% and 53±20% for the same temperature, in all cases air velocity was equal to 2 m $s^{-1}$ without any assisting drying technology (Colucci *et al.* 2018).

First, from the results in Table 1, under any comparable operative temperature condition, the VFD process required expressively less time compared to the AFD alone. Regarding product quality, ascorbic acid seems to be the most sensitive to AFD compared to VFD. The AA average percentage

reductions found for VFD are less than half of the ones found for AFD. Remembering that in the AFD a dried air flux runs over the product. This air flux could be considered as one of the major responsibles for the AA concentration drop observed in the AFD, since oxygen percentage would be greater compared to VFD and could degrade ascorbic acid. On the other hand, during VFD, the product was subjected to vacuum, so AA degradation pathways must have been anaerobic. In most cases, the rate constants for anaerobic degradation of ascorbic acid are two to three orders of magnitude lower than those for the oxidative reaction (Fennema 1996, Dennison and Kirk 1978). Additionally, as above-mentioned for AA content loss, drying time might play an important role and the AFD drying times compared were larger than the VFD ones.

For the phenolic compounds and antioxidant capacity, both processes seemed to have a similar effect on their content reduction, which suggests that both processes could be used with similar results. Correlations between the TPC and AC content variations after freeze-drying processes have been found in other studies (Izli *et al.* 2017, Shofian *et al.* 2012). Food matrixes are complex and several sinergistic and antagonistic interactions may happen simultaneously during a freeze-drying process affecting product quality in different ways. TPC was found to increase and decrease in different studies without following a specific trend while diverse explantions were given for each case like oxidative and enzymatic reactions (Harguindeguy and Fissore 2019). Binding to other compounds dificulting their measurement was also proposed as an explanation for TPC content reduction (Izli *et al.* 2017).

## 4. Conclusions

Chamber pressure seemed to have little influence on process duration in a VFD process, while temperature had an expressive effect on it. Regarding the nutritional properties, shelf temperature had a significant effect only on the antioxidant capacity of eggplants ($p$-value $< 0.05$), but it had no significant effect on the AA, neither the TPC losses observed after freeze-drying. An increase in AC loss was observed on samples dried under lower temperatures, but this effect might be related to longer process duration under these conditions. Pressure had no significant effect neither for AA nor for AC content loss but had a significant effect on TPC ($p$-value $< 0.05$). Under the tested conditions, TPC loss on samples was higher under lower operating pressures. Since drying time was found to be influenced only by temperature, this higher TPC loss observed seems to be influenced by the lower operational pressure itself rather than process duration. Comparing VFD and AFD effects on the content loss of targeted bioactive compounds of dried eggplants, ascorbic acid seemed to be the most sensible to AFD whereas the effect on TPC and antioxidant capacity contents seemed not as relevant.

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